Metabolic and genetic determinants of HDL metabolism and hepatic lipase activity in normolipidemic females

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Abstract The metabolic and genetic determinants of HDL cholesterol (HDL-C) levels and HDL turnover were studied in 36 normolipidemic female subjects on a whole-food low-fat metabolic diet. Lipid, lipoprotein, and apolipoprotein levels, lipoprotein size, and apolipoprotein turnover parameters were determined, as were genetic variation at one site in the hepatic lipase promoter and six sites in the apolipoprotein AI/CIII/AIV gene cluster. Menopause had no significant effect on HDL-C or turnover. Stepwise multiple regression analysis revealed that HDL-C was most strongly correlated with HDL size, apolipoprotein A-II (apoA-II), and apolipoprotein A-I (apoA-I) levels, which together could account for 90% of the variation in HDL-C. HDL size was inversely correlated with triglycerides, body mass index, and hepatic lipase activity, which together accounted for 82% of the variation in HDL size. The hepatic lipase promoter genotype had a strong effect on hepatic lipase activity and could account for 38% of the variation in hepatic lipase activity. The apoA-I transport rate (AI-TR) was the major determinant of apoA-I levels, but AI-TR was not associated with six common genetic polymorphism in the apoAI/CIII/AIV gene cluster. A simplified model of HDL metabolism is proposed, in which A-I and apoA-II levels combined with triglycerides, and hepatic lipase activity could account for 80% of the variation in HDL-C.—De Oliveira e Silva, E., M. Kong, Z. Han, C. Starr, E. M. Kass, S-H. H. Juo, D. Foster, H. M. Dansky, M. Merkel, K. Cundey, E. A. Brinton, J. L. Breslow, and J. D. Smith. **Metabolic and genetic determinants of HDL metabolism and hepatic lipase activity in normolipidemic females.** ***J. Lipid Res.*** **1999.** 40: **1211–1221.**

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HDL cholesterol (HDL-C) levels are inversely associated with the risk for developing cardiovascular disease in westernized cultures (1, 2). Environmental factors, such as diet, obesity, and exercise as well as genetic factors and gender influence HDL-C levels, with the genetic component estimated to account for ~40–80% of the interindividual variation in HDL-C levels (3–5). Both metabolic and genetic studies in humans have probed for the mechanisms that determine the variation in HDL-C levels. In an attempt to minimize the environmental factors, HDL metabolic studies have been performed on human subjects studied as inpatients fed defined diets. ApoA-I and apoA-II account for approximately 70% and 20% of HDL protein mass, respectively, and thus have been the focus of these metabolic studies. Several apoA-I and apoA-II turnover studies in human subjects selected for high and low levels of HDL-C have demonstrated that the interindividual variation in HDL-C levels is associated with an inverse correlation between HDL-C levels and apoA-I fractional catabolic rates (AI-FCR) primarily, and secondarily with apoA-II fractional catabolic rates (AII-FCR) (6–11). However, other studies have also found that the interindividual variation in HDL-C levels were associated with a positive correlation between either apoA-I levels or HDL-C and apoA-I transport rates (AI-TR) (12–14). The increases in HDL-C brought about by either increased dietary fat or by ethanol consumption have been found to be associated with increases in both AI-TR and apoA-II transport rate (AII-TR) (8, 15).

The genetic regulation of HDL-C levels has not been amenable to segregation analysis which suggests that

Abbreviations: HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; VLDL-C, very low density lipoprotein cholesterol; apo, apolipoprotein; AI-TR, apoA-I transport rate; AI-FCR, apoA-I fractional catabolic rate; AII-TR, apoA-II transport rate; AII-FCR, apoA-II fractional catabolic rate; BMI, body mass index; PBS, phosphate-buffered saline; HRP, horseradish peroxidase.

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HDL-C levels may be regulated by several genes and environmental factors in different populations, as expected for an oligogenic or polygenic trait (16). Cohen and his colleagues (17) successfully applied the sib-pair linkage analysis method to analyze the genetics of HDL-C variation. They determined that allelic variation at the hepatic lipase gene locus and the apoAI/CIII/AIV gene locus accounted for 25% and 22% of the interindividual variation in HDL-C levels, respectively (17). Cohen's laboratory subsequently identified a novel haplotype of the hepatic lipase gene (*LIPC*) consisting of 4 single base pair changes in an ~500 base pair region of the promoter that are in complete linkage disequilibrium (18). Genetic variation of the hepatic lipase promoter was associated with HDL-C levels in males, but not in females, such that the less common allele, with a 15% allele frequency, was associated with higher levels of HDL-C (18). The polymorphism at one of these four promoter sites was independently discovered and the less common allele was found to be associated with decreased hepatic lipase activity in a cohort of males with coronary artery disease (19). Two studies of Finnish subjects confirmed the effect of this hepatic lipase promoter polymorphism on both hepatic lipase activity (20) and HDL-C levels (21), and this latter effect was apparent in both males and females (21). While the frequency of the less common hepatic lipase promoter allele in American and European Caucasians ranges from 15% to 25% (19–21), in African Americans the frequency of this allele is 52%, and the increased frequency of this allele is associated with decreased hepatic lipase activity in African Americans (22). These prior studies regarding the hepatic lipase promoter polymorphism have been performed on outpatient subjects, without controlling for dietary or other environmental factors.

In regard to the apoA-I locus, there have been conflicting studies concerning the effect of the apoA-I −76 and +83 base pair polymorphisms on HDL-C levels. We previously reported a small (11%) effect of the −76 base pair polymorphism on AI-TR in a group including normolipidemic and hyperlipidemic subjects, without any effect on HDL-C levels (23).

The aim of the present study was to analyze the metabolic and genetic determinants on HDL metabolism in a cohort of females who were selected for normal LDL-C and plasma triglyceride levels, and studied as inpatients while on a whole-food metabolic diet. HDL-C was strongly correlated with HDL size, and with apoA-II and apoA-I levels (indicators of HDL particle number). HDL size was inversely correlated with plasma triglycerides, hepatic lipase activity, and body mass index (BMI). The hepatic lipase genotype was found to have a strong effect on hepatic lipase activity, and to thus play a direct role in HDL metabolism and the regulation of HDL-C levels. While the apoA-I transport rate (AI-TR) was strongly correlated with apoA-I levels and thus also correlated to HDL-C levels, there were no significant associations of AI-TR with six common polymorphisms in the apoAI/CIII/AIV gene cluster.

MATERIALS AND METHODS

Study population

Normolipidemic female subjects were recruited to participate in a metabolic ward study of HDL metabolism. Some of the subjects were part of a twin study, and the older member of the twin pair was arbitrarily chosen for inclusion in this study. To exclude potential effects of markedly elevated LDL-C and triglycerides on HDL metabolism, only subjects with age adjusted levels of LDL-C and triglycerides < the 75th percentile were selected for inclusion in this study (24). Based upon this selection, 2 subjects of 38 were excluded. None of the subjects was taking estrogens as hormone replacement therapy or for birth control. There were three African Americans and two Asian Americans in the final group which consisted of 36 non-related female subjects, of whom 14 were menopausal.

Diets

The diet was designed using the USDA Nutrient Data Base to conform to the American Heart Association Step II diet containing 15% protein, 60% carbohydrate, and 25% fat, with a P/S ratio of 1.5, and 80 mg cholesterol per 1,000 Kcal. Meals were prepared by the nutrition staff of The Rockefeller University Hospital Clinical Research Center. The diets consisted of whole foods from common ingredients of known composition, weighed to the nearest 0.1 gram. The caloric requirement for each subject was estimated according to the Harris-Benedict equation with an adjustment for physical activity (25, 26).

ApoA-I and apoA-II turnover study

The turnover study was performed in accordance with a protocol approved by The Rockefeller University IRB and all subjects signed informed consent prior to starting the study. Subjects were equilibrated on the metabolic diet for 2 weeks, followed by another 2-week period on the diet during which the turnover studies were performed. ApoA-I and apoA-II were purified from heterologous HDL which was prepared by sequential ultracentrifugation from a single healthy donor whose plasma was screened for viruses and infectious organisms. Purification of apoA-I and apoA-II was performed by sequential FPLC chromatography with MonoQ and Superose12 columns (Pharmacia), as previously described (7). ApoA-I and apoA-II were iodinated with ^{125}I and ^{131}I, respectively, as previously described (7). The labeled proteins were sterilized by passage through a 0.22-μm filter, and the filtrate was checked for sterility by inoculation of culture medium, and for pyrogenicity by the limulus test. Ten μCi of ^{125}I-labeled apoA-I and 25 μCi of ^{131}I-labeled apoA-II (about 100 μg of each apolipoprotein) were mixed together in 2 ml of saline and injected i.v. After the injection of labeled apoA-I and apoA-II, blood samples were drawn at 10 min, 4 h, 12 h, 24 h, 36 h, 48 h, and then daily for 14 days. Plasma was prepared and 1-ml aliquots were used for the determination of the remaining ^{125}I-labeled apoA-I and ^{131}I-labeled apoA-II radioactivity. The plasma apoA-I and apoA-II decay curves were normalized to the 10-min sample and analyzed using the Matthews model (27). The model, fitted to each decay curve using the SAAM II software system (28), was used to estimate the FCR and its error. ApoA-I and apoA-II TRs were calculated as the product of the FCR, the plasma concentration, and the plasma volume (assumed to be 4.5% of the body weight) divided by the body weight.

Plasma lipid, lipoprotein, and apolipoprotein determinations

Plasma samples for lipid and lipoprotein measurements, anticoagulated with EDTA, were obtained after a 12-h overnight fast during the third and fourth weeks on the diet, on days 1, 3, 7, 10,

and 14 of the turnover study. We have previously shown, under metabolic ward conditions similar to the ones in this study, that alterations in dietary fat and cholesterol result in a new steady state plasma lipoprotein profile by day 15, with no drift between days 15 and 25 (29). The plasma lipid, lipoprotein, and apolipoprotein values used for analysis were the average of these five measurements for each subject. Total cholesterol and triglycerides were determined by enzymatic methods using Boehringer Mannheim reagents. Lipoprotein cholesterol levels were determined after serial ultracentrifugation as previously described (7). Total and HDL-cholesterol values were standardized by the Lipid Standardization Program of the Centers for Disease Control and Prevention supported by the National Heart, Lung, and Blood Institute. Lipid and lipoprotein measurements were done on fresh specimens. Aliquots of plasma were also stored at −70°C for subsequent apolipoprotein determinations. Human apoA-I levels were determined by an ELISA assay. Mouse anti-human apoA-I monoclonal antibody (Biodesign), diluted 1:300 in bicarbonate buffer, was applied to wells of Nunc Maxisorb ELISA plates and incubated overnight at 4°C. All subsequent incubations were performed at room temperature with gentle agitation. Wells were washed with PBS and blocked with casein blocker (Pierce) for 1 h. Serum standards (Incstar) and serum samples were diluted 1:800,000 in buffer A (casein blocker/0.5% Tween 20), and applied to the wells for 1 h. Plates were washed with buffer B (PBS/0.5% Tween 20) and a goat anti-human apoA-I polyclonal antibody (Biodesign), diluted 1:1000 in buffer A, was applied for 1 h. After washing, mouse anti-goat IgG linked to horseradish peroxidase (HRP) (Pierce) was applied for 1 h. HRP enzyme was detected by incubation with TurboTMB substrate (Pierce). The reaction was terminated with 1 m sulfuric acid, and the absorbance was measured at 450 nm on a SpectraMax plate reader (Molecular Devices). Human apoA-II levels were also determined by an ELISA assay. The assay was similar to the apoA-I ELISA with the following modifications. The capture antibody was a 1:100 dilution of a mouse anti-human apoA-II monoclonal antibody (PerImmune). Serum standards (Kamiya Biomedical) and serum samples were diluted 1:30,000 in buffer A. The secondary antibody was biotinylated goat anti-human apoA-II polyclonal antibody (Biodesign), diluted 1:1000 in buffer A. After washing, streptavidin-HRP (Pierce), diluted 1:1000, was applied for 1 h.

Lipoprotein size determinations

Average HDL, low density lipoprotein (LDL), and very low density lipoprotein (VLDL) sizes were determined by proton nuclear magnetic resonance (NMR) spectroscopy, performed by Dr. James Otvos (University of North Carolina, Raleigh, NC), as previously described (30, 31). The calibration of lipoprotein diameter ranges (in nm) with the NMR subclasses was determined by electron microscopy (VLDL, LDL) and polyacrylamide gradient gel electrophoresis (HDL). NMR-derived estimates of LDL size based on electron microscopy data are ~5 nm smaller than those determined by gradient gel electrophoresis. Average particle sizes (nm diameter) of VLDL, LDL, and HDL were determined by weighting the relative percentage of each subclass by its diameter.

Hepatic lipase activity assay

On day 11 of the test diet and 3 days before isotope injection, a postheparin plasma sample was obtained 15 min after an intravenous bolus injection of heparin (60 units per kg body weight). Plasma was stored in aliquots at −70°C until assay for hepatic lipase. Hepatic lipase activity was measured using a commercially available fluorometric assay (Progen, Heidelberg, Germany), which was adapted to a 96-well microtiter plate format. Duplicate or triplicate 10-μl samples were mixed with 200 μl substrate solution in microtiter plates on ice. The reaction plates were preincubated for 5 min at 37°C, and then a kinetic assay was performed at 37°C for 15 min in a fluorescence plate reader (fmax, Molecular Devices, Sunnyvale, CA), using 355 nm and 405 nm wavelength filters for emission and excitation, respectively. Activity, expressed as μmol free fatty acids released/hr per ml postheparin plasma, was determined based on the slope of the increase in fluorescence intensity over time, compared to varying amounts of a standard postheparin plasma source of known hepatic lipase activity. Hepatic lipase activity of this standard plasma was determined using radioactive triolein in a glycerol-based assay with antibody inhibition of LPL, as previously described (7).

Hepatic lipase promoter genotype assay

As there is a significant discrepancy about the hepatic lipase transcriptional start site, we have adapted the numbering system of Guerra et al. (18). To detect the G to A polymorphism at −250 bp, the restriction endonuclease DraI (cuts at −253 bp) was used after PCR amplification of the hepatic lipase promoter region from genomic DNA. To amplify a 666 base pair product, the sense primer at −603 base pairs was GGG GGA AGA AGT GTG TTT ACT CTA GGA TCA CC, and the antisense primer at + 63 base pairs was CAC AGG GGA CTT GTG TCC ATT TCT CCG. Three μl of genomic DNA was add to 22 μl of a PCR mix containing a PCR buffer, whose composition was previously described (32), with 0.15 μl of a mix of the two primers at 50 pmol/μl, and 0.20 μl of taq polymerase. PCR was performed by 35 cycles at 94°C for 30 sec, 60°C for 30 sec, and 72°C for 1 min. The product was digested with 2.5 μl of DraI for 2 h at 37°C. There is an invariant DraI site at position −141, thus the common "1" allele without the DraI site at −253 yielded bands of 462 and 204 base pairs, while the less common "2" allele yielded bands of 350, 204, and 112 base pairs. The digest was run on a 2% agarose gel and stained with ethidium bromide. The presence of the 462 and/or 350 base pairs bands were used diagnostically to assign the genotypes as 11, 12, or 22.

ApoA-I promoter genotype assay

To determine the haplotype of the apoA-I polymorphisms at −76 and +83 base pairs, a 472 base pair band was amplified by PCR using the 5′ primer at −240 base pairs GCC TGC AGC ACT CCC CTC CCG, and the 3′ primer at +232 base pairs GAA GGG CCG TGG GGG ACC TGG. The reaction contained 5 μl of genomic DNA and 45 μl of a PCR mix containing a PCR buffer, whose composition was previously described (32), with 0.5 μl of a mix of the two primers at 50 pmol/μl, and 0.30 μl of taq polymerase. PCR was performed by 35 cycles at 94°C for 30 sec, 66°C for 30 sec, and 72°C for 1 min. MspI (1.2 μl) was added directly to the tubes and incubated at 37°C overnight. The reaction was run on 4% agarose gels (NuSieve 3:1). MspI cuts at an invariant site at +40 base pairs and can distinguish the haplotype at both polymorphic sites. Haplotype 1 cuts at both −76 and +83 yielding diagnostic bands of 165, 149, and 115 base pairs; haplotype 2 cuts only at −76 yielding diagnostic bands of 192, 165, and 115 base pairs; and haplotype 3 cuts only at +83, yielding diagnostic bands of 280 and 149 base pairs. The fourth haplotype, without cutting at either −76 or +83 would give bands of 280 and 192 base pairs, but was not observed in the present or a previous study (33).

Other apoAI/CIII/AIV gene cluster genotype assays

The XmnI polymorphism 5′ of the apoA-I gene (34) was detected after PCR amplification of a 392 base pair DNA fragment, from −2673 to −2281 base pairs relative to the apoA-I transcription start site. The 5′ and 3′ primers were GGA AAC AGG GGC CTA CAC TGT G and GTC TGC AGC CTT TGC AGT CTG ATC, respectively. The PCR product was digested with XmnI and run

on a 2% agarose gel. The common allele was uncut by XmnI, and the less common allele was cut into 173 and 219 base pair bands. The SstI polymorphism in the 3′ untranslated region of the apoC-III gene was detected after PCR amplification of a 551 base pair fragment, as previously described (35). In the presence of the SstI site, the 551 base pair band was replaced with a 232 base pair and a 317 base pair band. The HinfI and the PvuII polymorphisms in the apoA-IV coding region were detected by modifications of a method previously described (36). A DNA fragment that encodes amino acids 328 to 370 was amplified with the 5′ primer AGG GAC AAG GTC AAC TCC TTC TTC AGC ACC and a mismatched 3′ primer CAG CTC TCC AAA GGG GCC AGC ATC TGC AC. The PCR was carried out in a buffer whose composition was described previously (32) by first denaturing at 94°C for 4 min, followed by 35 rounds of denaturing for 30 sec at 94°C, annealing for 30 sec at 67°C, and elongating for 30 sec at 72°C. The reaction mix was split in half for separate digestions with HinfI or PvuII. The products were resolved on 4% agarose gels (NuSieve 3:1). The presence of the HinfI site allows digestion of the original 129 base pair band into 60 and 69 base pair bands. The presence of the PvuII site allows digestion into 89 and 40 base pair bands.

Assay for hepatic lipase promoter activity

The hepatic lipase promoter from −900 to +63 base pairs was isolated by PCR amplification of genomic DNA from subjects homozygous for the 1 and 2 alleles, respectively. PCR was performed with the 5′ primer GGA GCT CCG CTC CTG GCC AGA AAT CTC TTC, and the 3′ primer described above for the hepatic lipase genotype assay. PCR was performed using Pfu polymerase (Stratagene), according to the manufacturer's specifications with the addition of 10% DMSO to the reaction, for 30 cycles at 94°C for 45 sec, 61°C for 1 min, and 72°C for 1 min. The PCR products were cut with SacI (site added to the 5′ end of 5′ primer) and XmnI, which cuts at position +30 base pairs of the hepatic lipase gene, before the first ATG codon. The resulting fragment was ligated to the pGL3 luciferase expression vector (Promega) which was digested with SacI and XmaI. The promoters were fully sequenced and confirmed the presence of polymorphisms at positions, −763, −710, −514, and −250 base pairs, without any other changes. Plasmid DNA was prepared by ultracentrifugation, banding twice in CsCl ethidium bromide gradients. HepG2 cells in 6-well dishes were co-transfected by the calcium phosphate precipitation method with 4.8 μg of a luciferase expression clone and 0.4 μg of a β-galactosidase expression vector pCH110. After overnight incubation with the DNA precipitate, the cells were shocked with 15% glycerol in PBS for 4 min at room temperature. On the following day, the cells were lysed in situ and assayed for luciferase and β-galactosidase activities using the Dual-Light assay reagents (Tropix), according to the manufacturer's protocol. The results are calculated as the relative luciferase / β-galactosidase activity, normalized to the luciferase activity of the promoterless pGL3 luciferase expression vector.

Statistics

Data were analyzed to determine whether they were normally distributed. If not, log transformation was applied to correct skewness and kurtosis. Univariate linear regression analysis was used to determine regression coefficients (r) and significance (P) in pair-wise analysis of 17 parameters. Significance of these univariate correlations is reported without Bonferroni's correction for multiple comparisons, as this correction is considered too conservative when greater than 4 independent comparisons are made. However, with 16 independent comparisons for each parameter, the probability of obtaining a false correlation with a P value of 0.05 is 56%. Thus, weakly significant correlations must be confirmed by other tests, such as multiple regression analysis. The degree of correlation for some parameters is provided as r^2, defined as the fraction of the total variance of Y that is "accounted for" by variation in the univariate X. The value of r^2 would be the same if X and Y were interchanged. Thus, causality cannot be implied based upon this statistic alone. Stepwise forward multiple regression analyses were performed for HDL-C, and the main covariates of HDL-C levels, and the results are given as R^2 values, the coefficients of determination. This represents the percent of variation in the dependent variable explained by the independent variables in the model. Unpaired two-tailed t-tests and ANOVA tests were performed to determine significant differences between two groups, or more than two groups, respectively. Significance was defined as $P \leq 0.05$. Statistics were performed with SPSS 7.5.2 statistical package (normal distribution analysis), NCSS 2000 statistical package (stepwise multiple regression), and GraphPad Prism 2.0 (all other analyses).

RESULTS

Lipid, lipoprotein, and HDL turnover levels in the cohort and effects of menopause and race

Table 1 shows the age, body mass index, as well as 15 different lipid, lipoprotein, and HDL turnover parameters that were ascertained from 36 normolipidemic female subjects studied for 4 weeks on a low fat diet (25% of total calories derived from fat) in a metabolic ward. The levels of triglycerides and hepatic lipase activity were skewed, and thus were log transformed to normalize the data for statistical analysis. Fourteen of the 36 subjects were menopausal. In order to determine whether menopause was related to any of the 17 parameters of Table 1, unpaired two-tailed t-tests were performed comparing the menopausal and premenopausal subjects, with significance defined as $P \leq 0.05$. Menopause was associated with significantly increased age, LDL-C, VLDL-C, total cholesterol, and log triglyceride levels, and with slightly decreased VLDL size. The increases in the levels of LDL-C, total cholesterol, and log triglycerides in menopausal subjects of this study agree with the known effects of increasing age on these parameters (24). Menopause had no significant effect on the levels of HDL-C, apoA-I, apoA-II, hepatic lipase activity, HDL size, or any of the HDL turnover parameters. The 3 African American subjects had significantly greater mean body mass index (BMI) (30.5 ± 8.5) than the 31 Caucasian subjects (22.2 ± 3.5, $P = 0.002$), but there were no other significant differences in any other measured parameter. Thus, further analysis of HDL metabolism and genetic markers included all subjects.

Parameters correlated with HDL-C levels

The 17 parameters of Table 1 were subjected to pairwise univariate regression analysis, without correction for multiple independent comparisons as described in Materials and Methods, and the significant regression coefficients are displayed in **Table 2**. Nine parameters correlated significantly with HDL-C levels. The two strongest correlations with HDL-C were with HDL size ($r = 0.84$, $P < 0.0001$) and apoA-I levels ($r = 0.69$, $P < 0.0001$), the latter of which can be considered a marker for HDL parti-

TABLE 1. Lipid, lipoprotein, and other characteristics of female subjects, and the effect of menopause on these parameters

Parameter	Units	n[a]	Average	SD	Range	Premenopause (average ± SD)	Menopause[b] (average ± SD)	*P* Value
HDL-C	mg/dl	36	60.8	17.5	41–111	58.0 ± 16.6	65.1 ± 18.5	ns
LDL-C	mg/dl	36	105.1	30.6	59–175	88.7 ± 19.0	131.0 ± 27.5	<0.0001
VLDL-C	mg/dl	36	20.4	5.6	11–40	18.2 ± 4.2	23.7 ± 6.0	0.003
TC	mg/dl	36	186.2	38.5	132–254	164.9 ± 24.8	219.8 ± 31.7	<0.0001
TG[c]	mg/dl	36	78.3	22.6	48–148	71.7 ± 19.0	88.6 ± 24.6	0.02
HDL size	nm	36	9.52	0.48	8.6–10.8	9.51 ± 0.54	9.53 ± 0.39	ns
LDL size	nm	36	21.09	0.46	20.1–21.8	21.08 ± 0.56	21.11 ± 0.26	ns
VLDL size	nm	36	42.5	5.8	36–54	44.0 ± 6.3	40.0 ± 3.9	0.04
HL[c]	μmol/ml/h	35	9.99	5.4	2.86–20.35	10.42 ± 6.51	8.67 ± 3.44	ns
apoA-I	mg/dl	36	105	25.8	63–156	104.5 ± 24.9	105.8 ± 2739	ns
AI-TR	mg/kg/d	33	13.82	4.6	7.31–24.6	14.13 ± 3.75	13.40 ± 5.67	ns
AI-FCR	pool/d	33	0.296	0.067	0.136–0.461	0.310 ± 0.067	0.277 ± 0.064	ns
apoA-II	mg/dl	36	27.9	6	17–44	26.5 ± 5.0	30.1 ± 7.0	ns
AII-TR	mg/kg/d	35	2.42	0.69	1.55–4.04	2.42 ± 0.73	2.43 ± 0.65	ns
AII-FCR	pool/d	35	0.194	0.038	0.137–0.285	0.203 ± 0.037	0.18 ± 0.035	ns
BMI	kg/m^2	36	22.8	4.5	17.2–39.6	23.0 ± 5.1	22.5 ± 3.5	ns
Age	yr	36	39.9	17.7	19–86	27.9 ± 10	58.6 ± 7.9	<0.0001

Abbreviations not previously defined: TC, total plasma cholesterol; TG, plasma triglycerides; HL, hepatic lipase activity; ns, not significant.

[a] For n < 36, plasma samples not available or apoA-I or apoA-II iodination not successful.

[b] n = 14.

[c] Statistics performed on log 10 transformed data.

cle number. HDL-C is a significant fraction of total cholesterol in these normocholesterolemic women, and thus there was a strong correlation between these parameters ($r = 0.53$, $P = 0.001$). As expected, there was also an inverse correlation with the log of triglyceride levels ($r = -0.53$, $P = 0.001$). HDL-C was also significantly correlated, although less robustly, with LDL size ($r = 0.43$, $P = 0.01$) and apoA-II levels ($r = 0.35$, $P = 0.04$). Of the metabolic and turnover parameters, HDL-C was inversely correlated with AII-FCR ($r = -0.53$, $P = 0.001$), log hepatic lipase activity ($r = -0.50$, $P = 0.002$), and positively correlated with AI-TR ($r = 0.42$, $P = 0.01$). While HDL-C was not significantly correlated with AI-FCR ($r = -0.22$, $P = 0.23$), there was an inverse correlation with BMI ($r = -0.31$, $P = 0.06$), which almost reached statistical significance.

HDL metabolic relationships

As HDL-C was most strongly correlated with HDL size (and vice versa), the other parameters that correlated with HDL size were examined. The log of triglycerides has the next strongest, albeit inverse, correlation with HDL size ($r = -0.65$, $P < 0.0001$), and in fact the log of triglycerides correlated more strongly with HDL size than with HDL-C ($r = -0.53$, $P = 0.001$). Interestingly, the log of triglycerides did not correlate significantly with any of the HDL turnover parameters, or with the log of hepatic lipase activity, but the log of triglycerides did have an inverse correlation with apoA-I levels which was marginally significant ($r = -0.33$, $P = 0.05$). HDL size was also correlated with apoA-I levels ($r = 0.57$, $P = 0.0003$), although this may be an indirect correlation as both parameters are

TABLE 2. Univariate linear regression coefficient matrix

	HDL	LDL	VLDL	TC	Log TG	HDL Size	LDL Size	VLDL Size	Log HL	ApoA-I	AI-TR	AI-FCR	ApoA-II	AII-TR	AII-FCR	BMI	Age
HDL	1																
LDL	ns	1															
VLDL	ns	ns	1														
TC	0.53[c]	0.90[c]	ns	1													
Log TG	−0.53[c]	0.43[b]	0.67[c]	ns	1												
HDL size	0.84[c]	ns	ns	ns	−0.63[c]	1											
LDL size	0.43[b]	ns	ns	ns	−0.43[b]	0.63[c]	1										
VLDL size	ns	ns	ns	ns	ns	ns	−0.36[a]	1									
Log HL	−0.50[b]	ns	ns	−0.37[a]	ns	−0.57[c]	−0.46[b]	ns	1								
ApoA-I	0.69[c]	ns	ns	ns	ns	0.57[c]	0.41[a]	ns	ns	1							
AI-TR	0.42[b]	ns	ns	ns	−0.33[a]	ns	ns	ns	ns	0.75[c]	1						
AI-FCR	ns	ns	ns	ns	ns	−0.36[a]	ns	ns	0.50[b]	ns	0.60[c]	1					
ApoA-II	0.35[a]	ns	ns	0.40[a]	ns	ns	ns	ns	ns	−0.38[a]	0.38[a]	ns	1				
AII-TR	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.72[c]	1			
AII-FCR	−0.53[c]	ns	ns	−0.37[a]	ns	−0.52[b]	−0.40[a]	ns	0.48[b]	−0.40[a]	ns	ns	ns	0.63[c]	1		
BMI	ns	ns	ns	ns	ns	−0.46[b]	ns	ns	ns	ns	ns	ns	ns	ns	ns	1	
Age	ns	0.71[c]	0.51[b]	0.77[c]	ns	ns	ns	−0.38[a]	ns	ns	ns	ns	0.33[a]	ns	−0.34[a]	ns	1

[a] $P \leq 0.05$; [b] $P < 0.01$; [c] $P < 0.001$.

more strongly correlated with HDL-C levels. BMI was also inversely correlated with HDL size ($r = -0.46$ $P = 0.004$); besides this correlation and the marginally significant correlation with HDL-C (above), there were no other significant correlations with BMI in this study. Three other correlations with HDL size were, from strongest to weakest, log hepatic lipase activity ($r = -0.57$, $P = 0.0004$), AII-FCR ($r = -0.52$, $P = 0.001$), and, less strongly, AI-FCR ($r = -0.36$, $P = 0.04$). These latter three variables were all related to each other as there were equally strong correlations between the log of hepatic lipase activity and both AII-FCR ($r = 0.48$, $P = 0.004$) and AI-FCR ($r = 0.50$, $P = 0.003$). That AI-FCR was not as strongly correlated with HDL size as AII-FCR and hepatic lipase activity is consistent with the lack of a correlation between AI-FCR and HDL-C, while HDL-C, HDL size, AII-FCR, and hepatic lipase activity were all positively or inversely correlated with each other.

Stepwise multiple regression analysis derived models for HDL metabolism

The results of the simple correlation analyses and their metabolic relationships above suggest that an unbiased systematic multiple stepwise regression may be useful for simplifying the relationships involved in HDL metabolism. This analysis is crucial to determine which of the observed pair-wise correlations above are truly meaningful and independent of each other. Multiple stepwise regression for HDL-C revealed that HDL size, which had the strongest univariate correlation with HDL-C, and the levels of apoA-II and apoA-I were independent covariates which together could explain 90% of the variation in HDL-C (**Table 3**, model 1). This and the following relationships are shown diagrammatically in **Fig. 1**. It is of interest that apoA-II levels were more strongly correlated with HDL-C than apoA-I levels in this multiple regression model, while apoA-I levels were more strongly correlated with HDL-C in the univariate model. This can be attributed to the independence of apoA-II levels with HDL size, while apoA-I levels were less independent as they correlated with HDL size. In order to identify which independent parameters are determinants of HDL size, multiple stepwise regression for HDL size was performed after eliminating HDL-C as a covariate. Eighty-two percent of the variance in HDL size could be then be explained by three independent parameters: log of triglycerides, log of hepatic lipase activity, and BMI, (Table 3, model 2). All of these factors were inversely correlated with HDL size in univariate analysis. The levels of apoA-II and apoA-I, the other independent variables affecting HDL-C, are in turn correlated with their respective transport rates, as demonstrated in the univariate regression analysis in Table 2. Converting these regression coefficients into r^2 values, 49% and 58% of the variations in apoA-II and apoA-I levels were accounted for by variation in AII-TR and AI-TR, respectively. Another multiple stepwise regression analysis was performed for HDL-C, which did not rely on the specialized measurement of HDL-size. In this case 80% of the variance of HDL-C was correlated with four covariates, apoA-I levels, the log of triglycerides, the log of hepatic lipase activity, and apoA-II levels (Table 3, model 5), which was almost as predictive as model 1 which directly used HDL size as a covariate. In this analysis the level of apoA-I was the strongest covariate of HDL-C. The next strongest HDL-C covariates were the log of triglycerides and the log of hepatic lipase activity, which are the covariates most strongly associated with HDL size.

TABLE 3. Multiple stepwise regression for HDL-C and HDL size

Model	Covariate	Cumulative R^2
1. For HDL-C levels	HDL size	0.75
	apoA-II	0.86
	apoA-I	0.90
2. For HDL size, eliminating HDL-C[a]	log TG	0.42
	log HL	0.74
	BMI	0.82
3. For HDL-C, eliminating HDL size and total cholesterol[b]	apoA-I	0.55
	log TG	0.66
	log HL	0.74
	apoA-II	0.80

All covariates are shown that both increased the cumulative R^2 value by ≥ 0.04 and whose significance as an independent variable was $P < 0.05$.

[a] This stepwise regression was limited to the factors that had significant univariate correlations with HDL size in Table 2, thus eliminating any potential factors that could be indirectly related to HDL size via an effect on HDL-C levels.

[b] Total cholesterol was omitted from this model because it led to finding a perfect HDL correaltion with the inclusion of TC, VLDL-C, and LDL-C. These are artificial correlations due to the use of a formula to calculate VLDL-C levels: VLDL-C = TC − (LDL-C + HDL-C) thus HDL-C can be determined from TC, LDL-C, and VLDL-C values.

Genetic determinants of hepatic lipase activity

The hepatic lipase promoter polymorphism at −250 base pairs was detected by digestion with DraI after PCR amplification of genomic DNA. Using the convention of "1" for the more common allele, and "2" for the less common allele, there were 23, 11, and 2 subjects with the 11, 12, and 22 hepatic lipase genotypes, respectively. This corresponds to an allele frequency of 20.8% for the less common allele in this predominantly Caucasian cohort, in line with the frequency determined in previous studies in American and European Caucasians. This distribution of genotypes was not significantly different than that predicted by the Hardy-Weinberg equilibrium ($\chi^2 = 0.195$, $P = 0.91$). The three African American subjects in this cohort were evenly divided among the three hepatic lipase genotype groups. Hepatic lipase values for the individual subjects are shown in **Fig. 2**. Hepatic lipase activity was significantly affected by the genotype such that the activity was 11.86 ± 5.58, 6.90 ± 2.48, and 3.91 ± 0.08 units for the 11, 12, and 22 genotype subjects, respectively (mean ± standard deviation). Thus, there was an ~3-fold difference in hepatic lipase activity between the subjects with the 11 and 22 genotypes. The hepatic lipase activities were skewed and upon log transformation to normalize the distribution, the genotype effect on activity was highly significant ($P < 0.001$ by ANOVA). By univariate linear regres-

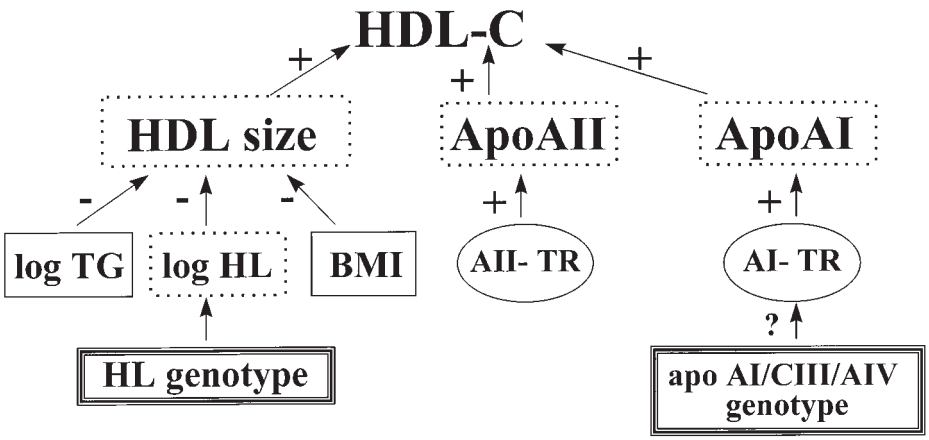

Fig. 1. Model of factors involved in the regulation of HDL-C levels. Plus and minus signs represent positive and inverse correlations, respectively; question mark refers to a speculated association. Solid-line boxes represent factors that are routine clinical measurements. Dotted-line boxes represent factors that can be measured from plasma samples, but are not routine clinical measurements. Ovals represent factors that can only be determined by turnover studies in human subjects. Triple-line boxes represent genetic information that can be ascertained from whole blood samples in a genetic laboratory; HL, hepatic lipase; TG, triglycerides.

sion analysis the hepatic lipase genotype could explain 38% (r^2) of the variation in the log of hepatic lipase activity ($r = 0.62$, $P < 0.0001$). Due to the demonstrated effect of hepatic lipase genetic variation on hepatic lipase activity, we have added this relationship to the model shown in Fig. 1.

Promoter activity of the two hepatic lipase alleles

In order to compare the relative promoter activity of the two hepatic lipase alleles, the proximal hepatic lipase promoter region was amplified from 11 and 22 homozygous subjects, and cloned into the pGL3 luciferase expression vector. The proximal promoter region used spanned from −900 to +30 base pairs, including all four promoter polymorphisms described by Cohen's laboratory (18).

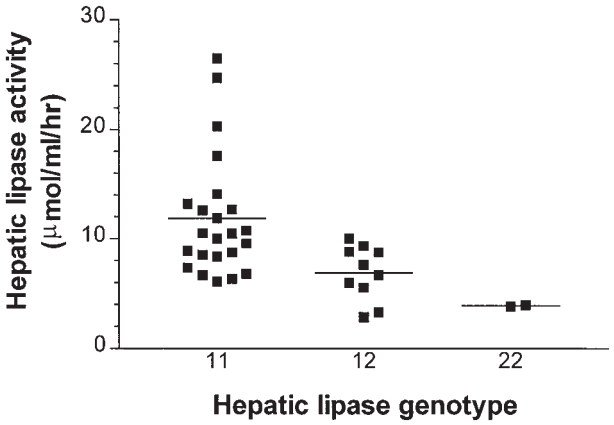

Fig. 2. Effect of hepatic lipase genotype on hepatic lipase activity. Postheparin plasma hepatic lipase activity was determined for each subject, as described in Methods section. The line shows the mean value for each hepatic lipase genotype group. Log hepatic lipase activities were significantly different among the three genotype groups, $P < 0.001$ by ANOVA.

The relative activities of the two allelic promoters were compared by transfection into HepG2 cells. The 1 and 2 alleles had equivalent, but weak, promoter activity, yielding only ~2-fold more activity than the promoterless pGL3 luciferase vector (n = 6 transfections for each allele and promoterless construction, data not shown).

Hepatic lipase genotype effect on physiological, lipid, lipoprotein, and turnover values

Although the data were limited by the occurrence of only two subjects who were homozygous for the hepatic lipase promoter 2 allele, a series of statistical analyses were performed to determine whether the hepatic lipase genotype was associated with any of the measured physiological, lipid, lipoprotein or turnover parameters. Using ANOVA analysis to compare the three hepatic lipase genotype groups, there was no significant effect of hepatic lipase genotype on age, BMI, log triglycerides, VLDL-cholesterol (VLDL-C), and LDL-cholesterol (LDL-C), VLDL size, apoA-I, AI-TR, AI-FCR, apoA-II, AII-TR or AII-FCR. There were significant hepatic lipase genotype effects on HDL-C, HDL size, and LDL size (**Table 4**), such that the subjects with the 22 genotype had more HDL-C, and larger HDL and LDL size. To get around potential artifacts of analyzing a group with two subjects, a *t*-test was used to look for the effect of the 2 allele by comparing the subjects with the 11 genotype to the subjects with the 12 and 22 genotypes. In this analysis the difference in HDL-C was no longer significant ($P = 0.31$); however, the differences in HDL size, LDL size, and AI-FCR were marginally significant ($P = 0.06$ for each, Table 4).

Genetic determinants of AI-TR

As AI-TR correlated with apoA-I and HDL levels in this study, and previous sib-pair analysis has demonstrated that the apoA-I gene locus has genetic linkage with HDL levels (17), six common polymorphisms in the apoAI/CIII/AIV

TABLE 4. Significant effects of hepatic lipase genotype on lipoprotein metabolism

HL Genotype	HDL-C	HDL Size	LDL Size	AI-FCR
	mg/dl	*nm*	*nm*	*pools/d*
11	58.5 ± 15.4	9.4 ± 0.4	21.0 ± 0.4	0.313 ± 0.066
12	60.1 ± 16.6	9.6 ± 0.4	21.2 ± 0.5	0.267 ± 0.065
22	90.0 ± 29.7	10.4 ± 0.5	21.8 ± 0.3	0.279 ± 0.049
ANOVA[a]	$P = 0.04$	$P = 0.008$	$P = 0.03$	NS
12 + 22	64.6 ± 20.7	9.7 ± 0.5	21.3 ± 0.5	0.269 ± 0.061
t-test[b]	NS	$P = 0.06$	$P = 0.06$	$P = 0.06$

Values given as mean ± standard deviation; NS, not significant; HL, hepatic lipase.
[a] Comparing 11, 12, and 22 genotypes.
[b] 11 vs. 12 + 22.

gene complex were tested for possible association with AI-TR. First the haplotypes of the common apoA-I polymorphisms at −76 and +83 base pairs were determined in order to ascertain if there were any associations with AI-TR. Only three of the four possible haplotypes were observed, which were designated 1 through 3 as described in the Methods section, with frequencies of 75%, 7.8%, and, 17.2%, respectively. Haplotypes 1 and 2 share the common allele at position −76 base pairs, with haplotype 2 having the less common allele at +83 base pairs. Haplotype 3 has the less common allele at the -76 base pair position, with the common allele at the +83 base pair position. Of the 33 subjects whose AI-TR rate data were determined, there were seventeen with the 11 genotype, four with the 12 genotype, eleven with the 13 genotype, and one with the 23 genotype. Using ANOVA analysis to compare the three genotype groups with more than one subject, there was no significant effect of the apoA-I promoter polymorphism on AI-TR, apoA-I levels, or HDL-C levels (**Table 5**). The 11 and 12 genotypes were pooled and compared to the 13 genotype in order to examine whether there was any effect solely of the polymorphism at −76 base pairs. By unpaired *t*-test analyses there were no significant differences between these two groups for AI-TR, apoA-I levels, or HDL-C levels (Table 5).

The XmnI polymorphism 5′ to the apoA-I gene was analyzed in a similar fashion, yielding 31 homozygotes for the common allele, 11 heterozygotes, and one homozygote for the less common allele, corresponding to an allele frequency of 19.7% for the less common allele. The SstI polymorphism in the 3′ end of the apoC-III gene, which is associated with hypertriglyceridemia (35), was also genotyped in this cohort of normolipidemic females yielding 29 subjects homozygous for the common allele, and 4 heterozygous subjects, which corresponds to a 6.1% allele frequency for the less common allele. Two polymorphisms in the apoA-IV gene were examined. For the PvuII polymorphism, there were 28 subjects homozygous for the common allele and 5 heterozygous subjects, which corresponds to a 7.6% allele frequency for the less common allele. For the HinfI polymorphism, there were 24 homozygotes for the common allele, 7 heterozygotes, and 2 homozygotes for the less common allele, which corresponds to a 16.7% allele frequency for the less common allele. Upon analysis by either unpaired *t*-test (2 genotype groups) or ANOVA (three genotype groups with more than 1 subject per group) there were no significant associations of any of these polymorphisms on AI-TR (data not shown). Whether there is any effect of genetic variation in the apoAI/CIII/AIV gene cluster on AI-TR remains to be determined, but as this is an intriguing possibility, it has been added to Fig. 1.

TABLE 5. Lack of significant effect of apoA-I promoter genotype on HDL metabolism

ApoA-I Genotype	n	AI-TR	ApoA-I	HDL-C
		mg/kg/d	*mg/dl*	*mg/dl*
11	17	13.7 ± 4.9	106 ± 24	58.8 ± 15.6
12	4	12.4 ± 2.9	94 ± 18	71.3 ± 8.3
13	11	14.9 ± 4.9	108 ± 32	60.4 ± 23.1
23	1	9.6	98	62
ANOVA[a]		$P = 0.64$	$P = 0.66$	$P = 0.47$
11 + 12	21	13.5 ± 4.5	104 ± 23	60.9 ± 15.2
t-test[b]		$P = 0.44$	$P = 0.62$	$P = 0.94$

Values given as mean ± standard deviation.
[a] Comparing 11, 12, and 13 genotypes.
[b] 11 + 12 vs. 13.

DISCUSSION

Understanding the regulation of HDL metabolism is an important goal, as HDL-C levels are strongly and inversely correlated with the risk for cardiovascular disease within developed populations. In this study, menopause had no effect on HDL-C levels or metabolism, which is consistent with the lack of an effect of aging on HDL-C levels in females who are not taking hormone replacement therapy (24). Hormone therapy does lead to a dramatic increase in HDL-C levels, but this pharmacological effect does not imply that menopause is associated with decreased HDL-C (24). The parameters that regulate HDL seem to vary in different studies according to the methods used and the subjects selected. Most likely, the factors that regulate both the turnover and production of apoA-I and apoA-II will be important in determining HDL-C levels. The lack of an association of HDL-C with AI-FCR observed in this study, but previously observed in several other studies (6), may be due to two factors (8–11). First, the current study selected only normolipidemic subjects while most other studies selected subjects with a broad range of HDL levels including many hyperlipidemic individuals with low HDL. For example the highest triglyceride and LDL-C levels of all subjects in the current study had levels that were below the mean levels of the female subjects studied by Brinton, Eisenberg, and Breslow (10). Gylling, Vega, and Grundy (14) have demonstrated that the AI-TR is the primary metabolic parameter correlated with apoA-I levels within each group of male subjects with either normal or low HDL-C levels, while differences in AI-FCR were found between the groups. Second, the subjects of this study were fed a lower fat diet and this may have an influence on the

relative role of AI-FCR and AI-TR in determining HDL-C levels. Brinton, Eisenberg, and Breslow (8) have found that the inverse association between AI-FCR and HDL-C levels was stronger in subjects on a high fat diet in than in the same subjects on a lower fat diet, while the positive association between AI-TR and HDL-C was stronger, although not significant in this group of 13 males and females, when the subjects were on a low fat diet.

Univariate linear regression analysis demonstrated that HDL size and apoA-I levels were the first and second strongest correlates of HDL-C levels in this study. This can be understood intuitively as the size and number of HDL particles determining HDL-C levels. HDL size alone could explain 75% of the variation in HDL-C, an extremely high r^2 value which may be in part due to the controlled environment and diet in this metabolic ward study. Stepwise multiple regression showed that HDL size and the levels of apoA-II and apoA-I could explain 90% of the variation in HDL-C. In turn, stepwise multiple regression showed that three inversely related covariates, triglycerides, hepatic lipase activity, and BMI, could explain 82% of the variation in HDL size. There are of course other plasma activities that can remodel HDL, including cholesteryl ester transfer protein (CETP), phospholipid transfer protein, and lecithin:cholesterol acyltransferase, which were not determined in the present study. We also propose a simplified multiple regression model for HDL-C levels that does not rely on direct measurement of HDL size. In this model variations in apoA-I levels, triglycerides, hepatic lipase activity, and apoA-II levels could explain 80% of the variation in HDL-C levels.

Focusing on the direct effect of hepatic lipase activity on HDL size, hepatic lipase in vitro can convert larger HDL into smaller HDL in a pathway associated with HDL catabolism and the loss of apoA-I (37). We thus speculate that variations in HDL size due to variations in hepatic lipase activity, triglycerides, and BMI may be causal in determining the rates HDL particle clearance. This could account for the inverse correlations of HDL size with both AII-FCR and AI-FCR in univariate regression analysis, and lead us to suspect that HDL-size influences apoA-I and apoA-II catabolism and not the other way around. In this study, 38% of the variance in hepatic lipase activity could be explained by a common genetic polymorphism in the hepatic lipase gene promoter. In this case causality of the promoter effect on hepatic lipase activity is biologically implied. Hepatic lipase activity and genotype both were associated with effects on HDL and LDL size, but not on VLDL size. This suggests that hepatic lipase acts more efficiently on the smaller lipoprotein substrates than on the larger VLDL substrate, and is consistent with the lack of a significant correlation observed between hepatic lipase activity and total plasma triglycerides in this study. The observed inverse relationship between triglycerides and HDL size may be mediated by CETP, as this inverse relationship is no longer observed in human subjects homozygous for CETP deficiency (38). Similarly, the inverse relationship with HDL size and BMI might be due to the adipose expression of CETP.

The current study was the first to examine the effects of the hepatic lipase promoter polymorphism on hepatic lipase activity and HDL-C levels in subjects studied in a metabolic ward to minimize environmental variation. The results confirmed previous studies showing that the less common allele is associated with decreased hepatic lipase activity (19, 20, 22). Thus, the hepatic lipase promoter genotype might serve as a useful surrogate for the hepatic lipase activity in studies of HDL metabolism where post-heparin plasma samples are not available. However, other unidentified factors are also important in determining hepatic lipase activity, as the promoter polymorphism was associated with less than half of the variation in hepatic lipase activity in this study. Although we observed a significant association of the hepatic lipase genotype on HDL-C levels when comparing all three genotype classes, the significance of this finding is limited by the occurrence of only two subjects homozygous for the rare allele, one of whom had very high levels of HDL-C. The genotype effect on HDL cholesterol was not significant when the carriers of the less common allele were pooled and compared with those homozygous for the common allele. However, the hepatic lipase genotype had a much stronger association with HDL size, consistent with the stronger correlation of hepatic lipase activity with HDL size than with HDL-C levels. The association of hepatic lipase genotype with HDL size was highly significant in comparing all three genotype classes, and marginally significant when the less common allele carriers were pooled. Thus, the hepatic lipase promoter genotype plays a direct role in hepatic lipase activity, and in turn may play an indirect role in the control of HDL metabolism, and possibly atherosclerosis susceptibility.

To examine whether differences in transcriptional activity of the two hepatic lipase promoter alleles could account for the observed difference in hepatic lipase activity, the promoter alleles, from −900 to +30 base pairs were cloned into a reporter gene expression plasmid and assayed by transfection into HepG2 cells. Although no difference between the alleles was observed in this assay, this was not a comprehensive assay of the hepatic lipase promoter, which is the subject of an ongoing study. Thus, it is possible that a transcriptional difference may still account for the observed genotype effect on hepatic lipase activity. First, the allelic variation that may lead to different transcriptional efficiencies may lay outside of region tested which included the four known allelic polymorphisms. And second, HepG2 cells expressed the reporter constructions weakly and may be depleted of transcription factors which are necessary for efficient expression of the hepatic lipase promoter and which may be involved in the discrimination between the two hepatic lipase promoter alleles.

As AI-TR had the strongest correlation with apoA-I levels and was also correlated with HDL-C levels in the current study, the apoA-I promoter variation at −76 and +83 base pairs along with four other common polymorphisms in the apoAI/CIII/AIV gene cluster were examined to see whether any of these genetic variations were associated with AI-TR. There was no association of any of these poly-

morphisms with AI-TR in these normolipidemic women. Previously, we had observed a small reduction in AI-TR associated with the −76 polymorphism, but that population consisted of both normal and hyperlipidemic subjects (23). While variation in the apoAI/CIII/AIV gene locus is likely to play a role in AI-TR and HDL-C metabolism, as 22% of the variation in HDL-C levels has been linked to variation at that locus (17), the site of the causative genetic variation remains to be discovered.

In conclusion, HDL-C levels were primarily a function of HDL size, apoA-II levels, and apoA-I levels. Of the HDL turnover parameters, AI-TR and AII-TR played the most important roles in determining apoA-I and apoA-II levels, respectively. In contrast to previous studies combining normolipidemic and hyperlipidemic subjects, AI-FCR was not correlated to apoA-I or HDL-C levels in these normolipidemic female subjects. HDL size was inversely related to hepatic lipase activity, BMI, and triglycerides. The hepatic lipase promoter polymorphism had a direct effect on hepatic lipase activity, which in turn was inversely correlated with variation in HDL size and HDL-C levels.

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